(2) *Related substances.* Proceed as directed in paragraph (b)(1) of this section for rifabutin content using the sample prepared as described in paragraph (b)(1)(i) of this section and calculating the amounts of related substances as follows.

(i) *Calculations.* Calculate the percentage of related substances as follows:

$$\text{Percent individual HPLC-related substance} = \frac{A_i \times 100}{A_t}$$

$$\text{Percent total HPLC-related substances} = \frac{A \times 100}{A_t}$$

where:

A_i = Area of the individual related substance peak;

A = The sum of areas of all peaks minus the area due to the rifabutin peak and solvent front peak; and

A_t = The sum of areas of all peaks in the chromatogram excluding the solvent peak.

(ii) [Reserved]

(3) *Dissolution test.* Proceed as directed in §436.215 of this chapter. The quantity (Q) (the amount of rifabutin activity dissolved) is 75 percent within 45 minutes.

(4) *Identity.* (i) The retention time of the rifabutin response in the HPLC procedure described in paragraph (b)(1) of this section as applied to the sample solution compares qualitatively to that of the rifabutin reference standard.

(ii) The identity of rifabutin capsules is also confirmed by the spectrophotometric identity test described in §436.370 of this chapter.

[59 FR 40808, Aug. 10, 1994]

Subpart C—Injectable Dosage Forms

§455.204 Aztreonam injectable dosage forms.

§455.204a Aztreonam for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Aztreonam for injection is a dry mixture of aztreonam and arginine. Its potency is satisfactory if each milligram of aztreonam for injection contains not less than 900 micrograms and not more than 1,050 micrograms of aztreonam when corrected for arginine content and moisture content. Its aztreonam immediate container fill (content) is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of aztreonam that it is represented to contain. It is sterile. It is nonpyrogenic. Its moisture content is not more than 2.0 percent. Its pH in an aqueous solution containing 100 milligrams of aztreonam per milliliter is not less than 4.5 and not more than 7.5. The aztreonam used conforms to the standards prescribed by §455.4a(a)(1), except if the aztreonam for injection is manufactured by lyophilization, in which case the aztreonam need not be sterile.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The aztreonam used in making the batch for potency, sterility, pyrogens, moisture, residue on ignition, heavy metals, and identity. If the aztreonam for injection is made by lyophilization, the aztreonam need not be tested for sterility.

(*b*) The batch for aztreonam potency, aztreonam content, sterility, pyrogens. moisture. and pH.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) The aztreonam used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency and content.* Determine both micrograms of aztreonam per milligram of sample and milligrams of aztreonam per container. Proceed as

directed in §436.361 of this chapter, except in addition to the column described in paragraph (a)(4) of that section, use a 5- to 50-centimeter saturator column having an inside diameter of 2 to 4.6 millimeters and packed with approximately 37 micrometer silica; and use the resolution test solution to determine resolution in lieu of the working standard solution. Perform the assay at ambient temperature, using an ultraviolet detection system operating at a wavelength of 206 nanometers, and a column packed with Chromegabond Diol (dihydroxypropane chemically bonded to porous silica), 5 to 10 micrometers or equivalent. Mobile phase, working standard solution, sample solution, resolution test solution, system suitability requirements, and calculations are as follows:

(i) *Mobile phase.* Acetonitrile:0.01*M* ammonium phosphate, pH 2.0. Transfer 1.15 grams of ammonium phosphate monobasic to a 1-liter volumetric flask. Add about 800 milliliters of distilled water and sonicate to aid dissolution. Adjust the solution to pH 2.0 with o-phosphoric acid, 85 percent. Dilute the solution to volume with distilled water and mix well. Transfer about 250 milliliters of this solution and 750 milliliters of acetonitrile to a suitably sized container and mix well.

(ii) *Preparation of working standard, sample, and resolution test solutions*—(*a*) *Working standard solution.* Transfer approximately 25 milligrams each of the aztreonam working standard and the arginine working standard, accurately weighed, to a 25-milliliter volumetric flask. Dissolve and dilute to volume with mobile phase (primary working standard solution). Further dilute with mobile phase to 0.2 milligram of aztreonam per milliliter (estimated).

(*b*) *Sample solutions.* Use separate containers for preparation of each sample solution as described in paragraph (b)(1)(ii)(*b*)(*1*) and (*2*) of this section.

(*1*) *Potency (micrograms of aztreonam per milligram).* Accurately weigh the container contents by difference and quantitatively transfer it to a 100-milliliter volumetric flask. Dissolve and dilute to volume with mobile phase. Further dilute in mobile phase to 0.2 milligram of aztreonam per milliliter (estimated).

(*2*) *Content (milligrams of aztreonam per container).* If packaged in containers with capacities of less than 100 milliliters, reconstitute the sample as directed in the labeling, using distilled water in lieu of reconstituting fluid. If packaged in bottles with capacities of 100 milliliters or greater, reconstitute with 10 milliliters of distilled water. Withdraw the total contents of each container or bottle and dilute with mobile phase to a concentration of 0.2 milligram of aztreonam per milliliter (estimated).

(*c*) *Resolution test solution.* Dissolve 10 milligrams of open ring aztreonam, [[(2-amino-4-thiazolyl)[(1-carboxy-1-methylethoxy)imino]acetyl]amino]-3-(sulfoamino)-butanoic acid, in 10.0 milliliters of primary standard solution. Further dilute 5 milliliters of this solution to 25.0 milliliters with mobile phase.

(iii) *System suitability requirements*—(*a*) *Tailing factor.* The tailing factor (*T*) of the aztreonam peak is satisfactory if it is not more than 2 at 5 percent of peak height.

(*b*) *Efficiency of the column.* The efficiency of the column (*n*) is satisfactory if it is greater than 1,000 theoretical plates.

(*c*) *Resolution.* The resolution (*R*) between aztreonam peak and open ring aztreonam is satisfactory if it is not less than 2.0.

(*d*) *Coefficient of variation.* The coefficient of variation (S_R in percent) of 5 replicate injections is satisfactory if it is not more than 2.0 percent.

If the system suitability requirements have been met, then proceed as described in §436.361(b) of this chapter. Alternate chromatographic conditions are acceptable provided reproducibility and resolution are comparable to the system. However, the sample preparation described in paragraph (b)(1)(ii)(*b*) of this section should not be changed.

(iv) *Calculations*—(*a*) *Potency (micrograms per milligram).* (*1*) Calculate the micrograms of aztreonam per milligram (uncorrected) as follows:

$$\text{Micrograms of aztreonam per milligram (uncorrected)} = \frac{A_u \times P_s}{A_s \times C_u}$$

where:

A_u=Area of the aztreonam peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);
A_s=Area of the aztreonam peak in the chromatogram of the working standard;
P_s=Aztreonam activity in the working standard solution in micrograms per milliliter; and
C_u=Milligrams of sample per milliliter of sample solution.

(*2*) Calculate the micrograms of arginine per milligram as follows:

$$\text{Micrograms of arginine per milligram} = \frac{A_u \times P_s}{A_s \times C_u}$$

where:

A_u=Area of the arginine peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);
A_s=Area of the arginine peak in the chromatogram of the working standard;
P_s=Arginine activity in the working standard solution in micrograms per milliliter; and
C_u=Milligrams of sample per milliliter of sample solution.

(*3*) Calculate the micrograms of aztreonam per milligram (corrected) as follows:

$$\text{Micrograms of aztreonam per milligram (corrected)} = \frac{\text{Micrograms of aztreonam per milligram (uncorrected)} \times 1{,}000}{1{,}000 - [\text{Micrograms of arginine per milligram} + (\text{Percent moisture}) \times 10]}$$

(*b*) *Content (milligrams of aztreonam per container).* Calculate the aztreonam content of the container as follows:

$$\text{Milligrams of aztreonam per container} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_u=Area of the aztreonam peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);
A_s=Area of the aztreonam peak in the chromatogram of the working standard;
P_s=Aztreonam activity in the aztreonam working standard solution in micrograms per milliliter; and
d=Dilution factor of the sample.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 50 milligrams of aztreonam per milliliter.

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 100 milligrams of aztreonam per milliliter.

[52 FR 4615, Feb. 13, 1987; 52 FR 8550, Mar. 18, 1987. Redesignated at 54 FR 40385, Oct. 2, 1989, and amended at 54 FR 41824, Oct. 12, 1989; 55 FR 11585, Mar. 29, 1990]

§ 455.204b Aztreonam injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Aztreonam injection is a frozen aqueous iso-osmotic solution of aztreonam and arginine. Each milliliter contains aztreonam equivalent to either 10 milligrams, 20 milligrams, or 40 milligrams. Its aztreonam content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of aztreonam that it is represented to contain. It is sterile. It is nonpyrogenic. Its pH is not less than 4.5 and not more than 7.5. It passes the identity test. The aztreonam used conforms to the standards prescribed by § 455.4(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on: